# MOLEBIO LAB #14: Bacterial Culture Techniques – Part III

## Introduction:

The number of cells in an overnight culture can be determined with a simple experiment involving the dilution of a bacterial culture. This will be carried out following Part II. We will also learn how to spread cells across a plate in this activity.

## Procedure:

### DILUTING A CULTURE

1. Set up five 1.5 mL microtubes, marked -2, -4, -6, -8, -10, with 990 μL of LB broth in each of them.
2. Label five LB plates by marking the bottom of them with -2, -4, -6, -8, -10 (and your group/class ID number on each: "G#")
3. Use a micropipettor with a sterile tip to add 10 μL of overnight culture to the first tube (labeled "-2"). Mix the tube thoroughly and use a fresh tip to transfer 10 μL of diluted cells from the -2 tube to the -4 tube. Mix thoroughly and transfer 10 μL from the -4 tube to the -6 tube, from the -6 tube to the -8 tube, and from the -8 tube to the -10 tube. USE A FRESH TIP EACH FOR EACH DILUTION!
4. Use a sterile tip for your micropipettor, lift the lid of a plate briefly to transfer 100 μL of diluted cells from the -10 tube onto an LB plate and spread across the plate (see steps 5 and 6). *Do not allow the suspensions to sit on the plates too long before proceeding through the next step.*

### STERILE SPREADING TECHNIQUE

Use either of the following methods to spread the cells:

5. Using a cell spreader…
    a. Dip the spreader into the ethanol beaker and *briefly* pass it through a Bunsen burner flame to ignite the alcohol. Allow alcohol to burn off *away from the flame*; the rod of the spreader will become too hot if left in the flame.

       **NOTE**: Be careful not to ignite the ethanol in the beaker! **DO NOT PANIC** if the ethanol is accidentally ignited. Cover the beaker with a Petri dish lid or other cover to cut off oxygen and rapidly extinguish the fire.

    b. Lift the lid of one plate just enough to allow spreading; *do not place lid on lab bench*.
    c. Cool spreader by gently rubbing it on the surface of the agar *away* from the cell suspension or by touching it to condensation on the plate lid.
    d. Touch the spreader to the cell suspension, and gently drag it back and forth several times across the surface of the agar. Rotate plate one-quarter turn, and repeat spreading motion. Try to spread the suspension evenly across agar surface. *Be careful* not to gouge the agar.
    e. Replace plate lid. Return cell spreader to ethanol **without flaming!**
    f. Repeat a) through e) for the remaining plates (-8, -6, -4, -2).
6. Using spreading beads…
    a. Lift the lid of one plate enough to allow adding the beads; *do not place the lid on the lab bench*.
    b. Carefully pour five to seven sterile glass spreading beads from a 1.5 mL tube onto the agar surface.

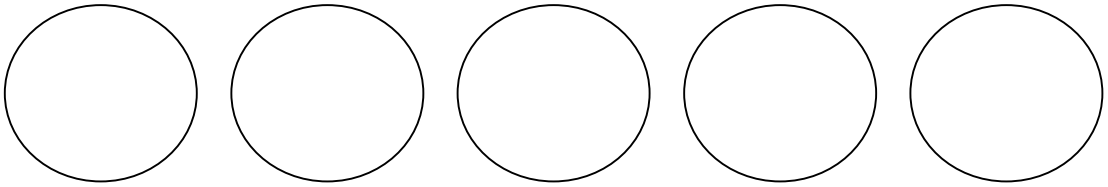
   c. Close plate lids and use a swirling motion to move glass beads around the entire surface of the plate. This evenly spreads the cell suspension on the agar surface. Continue swirling until the cell suspension is absorbed into the agar.

7. Stack, tape, and label your plates. Incubate the plates overnight and count the colonies. Each colony represents a single original cell that multiplied.
8. Take time for a responsible cleanup.
   a. Segregate for proper disposal bacterial cultures *and* tubes, pipettes, and micropipettor tips that have come into contact with the cultures.
   b. Disinfect overnight culture and pipettes and tips with 10% bleach solution, or disinfectant.
   c. Wipe down lab bench with soapy water, 10% bleach solution, or disinfectant.
   d. Wash hands (as always) before leaving lab.

Micklos, David A. Freyer, Greg A. *DNA Science*. 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2003. 331-350, 393-394. Print.

Name: _____

1. Based on the number of colonies/original cells counted and the dilution factor for each tube, calculate the concentration of the cells in the original culture. Start by indicating on each representation of a dish how many colonies were found. Then show your calculations below – for all dishes! (I know the numbers should ***should*** be the same – let us just check…)




HINT: If there were 15 colonies on the plate spread from the -8 tube, then there were 150 bacteria in the -8 tube (you removed 100 μL out of 1 mL – which is 1000 μL, a tenth of the total – so 15 colonies x 10 = 150 bacteria in the -8 tube). The -8 tube was a $10^8$-fold dilution of the original culture, so 150 bacteria x $10^8$ = 150 x $10^8$ or 1.5 x $10^{10}$ bacteria per milliliter in the original overnight culture.